

IN THE CLAIMS:

88. (Amended) A purified and [recombinant or] isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 and further comprising an amino-terminal methionine residue.
94. (Twice Amended) A method for the production of glial cell line-derived neurotrophic factor polypeptide comprising the steps of:
- (a) culturing a transformed or transfected host cell containing a nucleic acid sequence according to Claim 88;
  - (b) maintaining said host cell under conditions allowing the expression of glial cell line-derived neurotrophic factor polypeptide by said host cell; and
  - (c) optionally, isolating the glial cell line-derived neurotrophic factor polypeptide expressed by said host cell.
117. (Amended) A purified and isolated DNA [nucleic acid] sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence:
- (a) comprises nucleotides 304 through 705 of SEQ ID NO:3 or nucleotides 105 through 506 of SEQ ID NO:5; or
  - (b) encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; or
  - (c) encodes a polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment; or
  - (d) hybridizes [under conditions of reduced stringency] to a nucleic acid sequence complementary to those defined in (a), (b) or (c), under [wherein said] conditions comprising [include] hybridizing sequences in 6X SSPE and 0.1% SDS at 42 to 50°C followed by washing in 2X SSPE and 0.1% SDS at room temperature to 50°C; or

(e) encodes a polypeptide encoded by a nucleic acid sequence defined in (a), (b), (c) or (d) but differs in codon sequence due to the degeneracy of the genetic code; and wherein said polypeptide[encoded glial cell line-derived neurotrophic factor] has the capability to promote dopamine uptake in dopaminergic neurons.

118. (Amended) A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, said nucleic acid sequence comprising nucleotides 105 through 506 of SEQ ID NO:5, wherein said polypeptide [factor] has the capability to promote dopamine uptake in dopaminergic neurons.

119. (Amended) A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence as set forth in SEQ ID NO:6, wherein said polypeptide [factor] has the capability to promote dopamine uptake in dopaminergic neurons.

120. (Amended) A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment, and wherein said polypeptide [factor] has the capability to promote dopamine uptake in dopaminergic neurons.

121. (Amended) A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, comprising a sequence which hybridizes [under conditions of reduced stringency] to a nucleic acid sequence complementary to nucleotides 304 through 705 of SEQ ID NO:3 or nucleotides 105 through 506 of SEQ ID NO:5 under conditions comprising [those encoding an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6, wherein said conditions include] hybridizing said sequences in 6X SSPE and 0.1% SDS at 42 to 50°C followed by washing in 2X SSPE and 0.1% SDS at room temperature to 50°C, and wherein said polypeptide [factor] has the capability to promote dopamine uptake in dopaminergic neurons.

124. (Amended) A purified and isolated nucleic acid sequence according to claim 118 or 119 further comprising nucleotides encoding a pre-pro amino acid sequence as set forth in SEQ ID NO:26 [SEQ ID NO:25] amino acid residues 10 through 86 [1 through 77].

125. (Amended) A purified and isolated DNA [nucleic acid] sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence:

- (a) encodes a polypeptide comprising a [the] pre-pro form of human glial cell line-derived neurotrophic factor polypeptide as set forth in SEQ ID NO:26 [SEQ ID NO:25] amino acid residues 10 through 220 [1 through 211]; or
- (b) encodes a polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence set forth in SEQ ID NO:26 amino acid residues 10 through 220 [SEQ ID NO:25] when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment; or
- (c) hybridizes [under conditions of reduced stringency] to a nucleic acid sequence complementary to those defined in (a), under [wherein said] conditions comprising [include] hybridizing sequences in 6X SSPE and 0.1% SDS at 42 to 50°C followed by washing in 2X SSPE and 0.1% SDS at room temperature to 50°C; or
- (d) encodes a polypeptide encoded by a nucleic acid sequence defined in (a), (b) or (c) but differs in codon sequence due to the degeneracy of the genetic code; and

wherein said polypeptide [encoded glial cell line-derived neurotrophic factor] has the capability to promote dopamine uptake in dopaminergic neurons.

133. (Amended) A host cell according to claim 127 wherein said cell is transformed or transfected *ex vivo* and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

134. (Amended) A host cell according to claim 127 wherein said cell is suitable for human implantation and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

135. (Amended) A transformed or transfected host cell which expresses [recombinantly transformed or transfected to express] a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, said nucleic acid sequence operatively linked to a non-native promoter, wherein said nucleic acid sequence:

- (a) comprises nucleotides 105 through 506 of SEQ ID NO:5; or
- (b) encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; or

(c) encodes a polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment; and wherein said encoded polypeptide [glial cell line-derived neurotrophic factor] has the capability to promote dopamine uptake in dopaminergic neurons.

136. (Amended) A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence which is in excess of [at least] 90% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

141. (Amended) A host cell according to claim 138 wherein said cell is suitable for human implantation and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

142. (Amended) A host cell according to claim 138 wherein said cell is transformed or transfected *ex vivo* and wherein said cell expresses and secretes said glial cell line-derived neurotrophic factor polypeptide.

143. (Amended) A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a host cell according to claim 127 under conditions suitable for the expression of glial cell line-derived neurotrophic factor polypeptide; and optionally
- (b) isolating said glial cell line-derived neurotrophic factor polypeptide therefrom.

148. (Amended) A method according to claim 143 further comprising the step of refolding said glial cell line-derived neurotrophic factor polypeptide.

149. (Amended) A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a host cell transformed or transfected with a vector according to claim 126 [or 137] under conditions suitable for the expression of glial cell line-derived neurotrophic factor polypeptide; and optionally

- (b) isolating said glial cell line-derived neurotrophic factor polypeptide expressed by said host cell.

150. (Amended) A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising [a host cell containing] a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide [factor], wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence is selected from the group consisting of:

- (i) nucleotides 105 through 506 of SEQ ID NO:5; or  
(ii) nucleotides encoding a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; or  
(iii) nucleotides encoding a polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 when four gaps in a length of 100 amino acids may be introduced to assist in that alignment; and

- (b) isolating said expressed polypeptide [glial cell line-derived neurotrophic factor] in a substantially purified form from said host cell culture,

wherein said polypeptide [glial cell line-derived neurotrophic factor] has the capability to promote dopamine uptake in dopaminergic neurons.

152. (Amended) A method [according to claim 150] for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence encodes a polypeptide [factor] comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6; and

- (b) isolating said expressed polypeptide in a substantially purified form from said host cell culture.

wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

153. (Amended) A method [according to claim 150] for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

(a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said nucleic acid sequence encodes a polypeptide [factor] comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment; and

(b) isolating said expressed polypeptide in a substantially purified form from said host cell culture,

wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

155. (Amended) A method according to claim 150 further comprising refolding expressed glial cell line-derived neurotrophic factor polypeptide to form a disulfide-bonded dimer.

156. (Amended) A method according to claim 150 wherein said glial cell line-derived neurotrophic factor polypeptide is expressed by a bacterial host cell and is refolded to form a disulfide-bonded dimer.

157. (Amended) A method according to claim 150 wherein the expressed glial cell line-derived neurotrophic factor polypeptide is secreted by said host cell.

Please add the following claims:

--159. A transformed or transfected host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence comprises nucleotides 105 through 506 of SEQ ID NO:5, operatively linked to a non-native promoter; and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

160. A transformed or transfected host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6, wherein said nucleic acid sequence is operatively linked to a non-

native promoter, and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

161. A transformed or transfected host cell which expresses a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide, wherein said nucleic acid sequence encodes a polypeptide comprising an amino acid sequence which is in excess of 70% identical to an amino acid sequence set forth in SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment, wherein said nucleic acid sequence is operatively linked to a non-native promoter, and wherein said encoded polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

162. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence which is in excess of 95% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

163. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence which is in excess of 99% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

164. A purified and isolated nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide comprising an amino acid sequence which is in excess of 80% identical to an amino acid sequence of SEQ ID NO:4 or SEQ ID NO:6 when up to four gaps in a length of 100 amino acids may be introduced to assist in that alignment and wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

165. A method for the production of glial cell line-derived neurotrophic factor polypeptide, comprising the steps of:

- (a) culturing a transformed or transfected host cell comprising a nucleic acid sequence encoding a glial cell line-derived neurotrophic factor polypeptide under conditions suitable for the expression of said polypeptide, wherein said nucleic acid sequence is

operatively linked to a non-native promoter, and wherein said nucleic acid sequence comprises nucleotides 105 through 506 of SEQ ID NO:5; and

- (b) isolating said expressed polypeptide in a substantially purified form from said host cell culture,

wherein said polypeptide has the capability to promote dopamine uptake in dopaminergic neurons.

166. A method according to claim 165 wherein said host cell is an animal cell or microorganism.

167. A method according to claim 165 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

168. A method according to claim 165 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.

169. A method according to claim 152 wherein said host cell is an animal cell or microorganism.

170. A method according to claim 152 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

171. A method according to claim 152 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.

172. A method according to claim 153 wherein said host cell is an animal cell or microorganism.

173. A method according to claim 153 wherein the expressed glial cell line-derived neurotrophic factor is secreted by said host cell.

174. A method according to claim 153 further comprising the step of refolding expressed glial cell line-derived neurotrophic factor to form a disulfide-bonded dimer.

175. A host cell comprising *E. coli* transformed or transfected with a nucleic acid sequence according to Claim 88.